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THE NEMATOCYST OF HYDRA (Part IV).

THE INHIBITION OF THE NEMATOCYST FORMATION PROCESS IN HYDRA

by

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INTRODUCTION.

In 1955 BRIEN, subjecting hydra to X-rays, was able to selectively destroy interstitial cells without interfering with the metabolism of other cell types. These irradiated forms were, of course, not able to manufacture nematocysts and died a few weeks after irradiation since they were not able to feed or to effect tissue repair.

In the present set of experiments, it was desired to produce a « nematocystless » animal, in order to study the migration pathways of the nematocysts. However, it was also desired to suppress nematocyst formation without employing such drastic procedures as X- irradiation, for an irradiated animal is not

able through processes of cell de-differentiation to form interstitial cells again. For this study it was desired to find a process whereby it was possible to suppress formation of nematocysts for a given period of time, and then, by removing the blocking agent, to allow for further production of this organelle.

A relatively simple technique was devised whereby it was possible, through grafting procedures involving hydra of different species, to inhibit the formation of nematocysts, and at any desired time cause the process of nematocyst formation to be initiated again. This paper will report on this technique, and will also include a short discussion of the mechanisms involved in the inhibition process.

Materials and Methods.

Two species of hydra were employed in the following experiments. The first species, *Pelmatohydra oligactis* (Pallas) was reared in general by the methods described previously in the present series (See *The Nematocyst of Hydra,* Part I). The second species was discovered recently in Belgium by Dr. Paul Brien and has not been named at the present time. A paper dealing with the taxonomy of this species will be published during the coming year; for the present it will be classified as Species X.

Most of the grafting procedures described in this paper were of two types. One type consisted of the distal portion (hypostome, tentacles and growth region) of Species X grafted to the proximal portion (stomach region, budding region, peduncle and basal disk) of *oligactis.* The second grafting procedure was a reverse of the one just described, i.e., that proximal portion of Species X to the distal portion of *oligactis.* Other grafting procedures will be described as they are dealt with in this paper.

Several animals were also prepared for histological study. Most of the animals were examined as whole mounts and stained with methylene blue at p.H 7. This procedure for the staining of whole hydra is described in Part II of the present series. Animals which were prepared for sectioning were fixed in Bouin's solution and stained with iron hematoxylin, light green, and phloxine. This technique proved to be excellent for studying the developmental stages of nematocysts.

Usually, when two different species of hydra are grafted to one another it is necessary to vitally stain one of the species in order to recognize the grafted portions from one another. It

was not necessary to do this in the present investigation because there are several obvious differences, both macroscopic and microscopic, which serve to differentiate the two species.

A few of these differences will be outlined very briefly. The average length of *oligactis* as it rests normally in a finger bowl is roughly 3-6 mm. Species X, on the other hand, is often as large as 5 cm. The diameter of the new species is also quite different from that of *oligactis*. When one attempts to graft the two species by threading them on a human hair, he will often find that the *oligactis* portion will pass into the gastrovascular cavity of the new species. Since Species X has a diameter much larger than that of *oligactis*, there is always a very noticeable constriction at the point where the grafted portions are joined. The most obvious feature which serves to distinguish the two species is pigmentation. Both species during the course

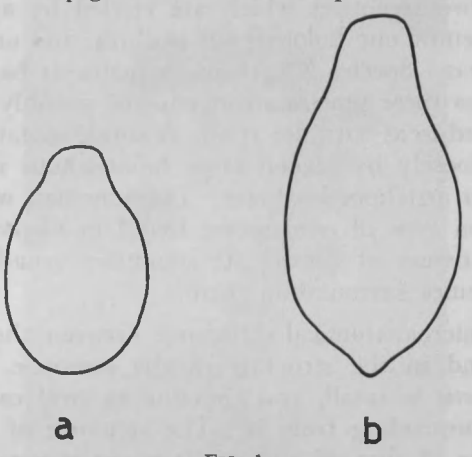

FIG. 1.
A schematic illustration showing the differences between the stenoteles of Species X and *Pelmatohydra oligactis*.
a. Pelmatohydra oligactis. - B. Species X.

of these experiments were fed with the brine shrimp, *Artemia salina*. However, after feeding, the tissues of *oligactis* are a bright orange, while those of Species X are brown. The reason for this color difference is not known at the present. An examination of whole mounts of Species X reveals dense, brown, non-refractile bodies as large as 50 microns in the digestive cells of the gastrodermis (See Fig. 2). *P. oligactis*, on the other hand contains small, orange aggregations of carotenoid crystals in its digestive cells (See BURNETT, 1959). Therefore, one can recognize

the two species after they have been grafted to one another by simply looking at their color; no magnifying device is needed for this recognition.

A final observation which will readily distinguish between the two species will be discussed more fully in the following paper. For the present, it will simply be mentioned that the tentacles of Species X are short, thick, and are many times smaller in length than the body of the animal. The tentacles of *oligactis* are very long, many times longer than the body of the animal, are extremely thin, and are often 5 times as long as those of Species X.

Microscopic recognition of the two species is also very simple. The most obvious difference is found in the nematocyst batteries. The battery of *oligactis* is very precisely arranged; there are one or two stenoteles which are circled by approximately nine desmonemes, one holotrichous isorhiza, and one or no atrichous isorhizas. Species X contains nematocyst batteries which do not possess these precise arrangements, possibly they should not be referred to as batteries at all. A single stenotele is usually surrounded loosely by several large holotrichous isorhizas and a few smaller atrichous isorhizas. Desmonemes, which are the most common type of nematocyst found in *oligactis* are more rare in the tissues of *Species X*; stenoteles usually have only two desmonemes surrounding them.

Another microanatomical difference between the two species may be found in the structure of the stenotele. The stenotele of *oligactis* is small, and contains an oval capsule with a small neck protruding from it. The stenotele of Species X is twice the size of that of *oligactis* (average stenotele of Species X equals 22 microns from base to neck), is pyriform rather than oval in shape, and contains a large « neck » protruding from the capsule. Also, there is a small nipple, lacking in *oligactis*, projecting from the end of the capsule opposite the operculum. Figure 1 illustrates these differences schematically.

Therefore in a matter of seconds an investigator can distinguish these two species both by macroscopical and microscopical examination. To the author's knowledge, Species X has never been described in the United States. If upon the author's return to the United States, these animals are successfully cultured, the author will gladly furnish them to anyone interested in studying this mammoth species.

Results and Conclusions.

Forty proximal portions of *Pelmatohydra oligactis* were grafted to an equal number of distal regions of Species X. On the 3, 5 and 6th day following the graft several animals were prepared for whole mounts. The tentacles of 8 animals examined after a three day period appeared to be normal in every respect as far as nematocyst distribution was concerned. However, 5 animals examined closely under an oil immersion objective revealed that there were fewer nematocysts (stenoteles) in the growth region than there were in normal forms. Also there was a definite depletion of nematocysts in the tentacle bases.

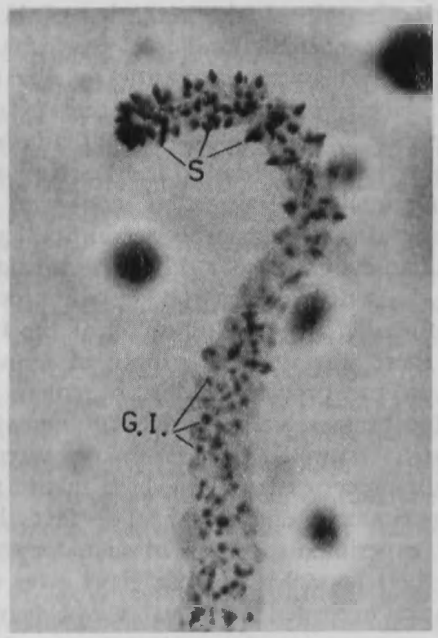

Fig. 2.

Photograph of a whole tentacle of Species X which has been grafted to the proximal portion of *Pelmatohydra oligactis* for a period of six days. Note the presence of nematocysts only in the extremeties of the tentacle. Also note the large crystalline bodies in the gastrodermis of Species X. S : stenotele; G.I. : gastrodermal inclusion.

Animals which were examined on the 5th day revealed that approximately 6 animals, though having a rather reduced stenotele supply, had a uniform distribution of nematocysts along

the length of the tentacle. Seven animals, however, lacked nematocysts completely in the lower ½ region of the tentacle. Occasionally, five or six stenoteles were sighted in these lower regions, but a normal hydra would be expected to contain at least 100 stenoteles in the same region. It must be stressed that during this examination attention was paid strictly to the nematocysts characteristic of Species X.

Seven animals examined on the 6th day following the graft contained nematocysts only in the distal ¼ region of the tentacles (See Figure 2). The growth regions of two of these animals contained no nematocysts whatsoever. The remaining 5 animals contained approximately 4-8 stenoteles. Four animals had a rather sparse, but uniform, distribution of nematocysts along the entire length of the tentacle, while two animals, though containing a normal number of isorhizas, contained no stenoteles except at the very extremities of the tentacles.

Clearly, the grafting of *oligactis* to Species X resulted in the inability of Species X to form its normal number of nematocysts. The fact that there was a progressive loss of nematocysts in the proximal-distal direction along the length of the tentacles indicates that growth processes in Species X were functioning normally. As epidermal cells were pushed towards the extremities of the tentacles through growth processes, nematocysts were continually being sloughed off at the tips of the tentacles. Since there was no new supply of nematocysts being manufactured in the growth region to replace this loss, the tentacles became progressively deprived of nematocysts. Semal-Van Gansen (1951) showed that the time required for a cell to move by growth processes from the base to the tip of the tentacle was approximately one week. The fact that the hydra in the present experiments contained nematocysts only in the extremities of their tentacles after six days gives strong support to the observation recorded by Semal-Van Gansen.

The fact that some animals continued to make nematocysts for six days following the graft is not explainable at the present time. Preliminary observations suggest that the physiological state of *Pelmatohydra oligactis* greatly determines whether nematocyst inhibition will be effected in Species X. When nematocyst inhibition was most apparent, the *oligactis* involved in the graft had been recently fed and was in a « robust » condition. When inhibition was not effected the *oligactis* were usually pale in color, hence, undernourished. Furthermore, it

is difficult to explain why in two cases only stenotele production was inhibited in Species X while the count of isorhizas remained normal.

In order to determine whether the inhibition of nematocyst formation was permanent or temporary, 5 hydra (Species X) were separated from *oligactis* at the point of junction of the grafted portions six days after the graft was performed. These 5 animals were selected from a group of 20 grafted animals because, with the aid of a binocular dissecting microscope, it was possible to see that they contained nematocysts only in the extremities of their tentacles. Four days following the separation of the grafted portions there was a prompt renewal of nematocyst formation, and after two additional days the tentacles appeared normal in every respect.

Another group of 5 animals in which nematocyst formation had been inhibited in Species X were also separated at the point of junction of the two grafts. These distal portions of Species X were then regrafted to normal trunks (stomach region, budding region, peduncle, and basal disk) of the same species. Two days following the regrafting, the tentacles of the animals were filled with nematocysts of all four varieties. These nematocysts, by necessity, did not arrive in the tentacles through the normal growth processes of the animal; it is obvious that they arrived in the nematocyst batteries of the tentacles only after an active migration via the gastrovascular cavity. However, the authors do not completely rule out the possibility that the cnidoblast cells were capable of moving in an amoeboid fashion to the tentacles along the base of the epidermis. There is no concrete evidence that these nematocysts passed out into the gastrovascular cavity before reaching the tentacles.

The results of the foregoing experiments indicate that a substance or substances released from the tissues of *oligactis* are capable of suppressing the formation of nematocysts in Species X. The fact that the inhibition ceases once the grafted portions are separated lends support to the idea that the inhibition is *humoral* in nature and that a constant supply of the inhibitory substance is necessary in order for a decrease in nematocyst production to be evidenced. Unfortunately, the mechanism of this inhibition remains a mystery at the present time. It may be mentioned that whenever inhibition is evidenced, there are usually several dozen nematocysts sighted in the digestive

cells of the gastrodermis. These nematocysts are always in a state of semi-degradation. Perhaps, the cnidoblast cells of Species X are able to begin nematocyst formation but are unable to complete the process. These abnormal cnidoblasts may pre-

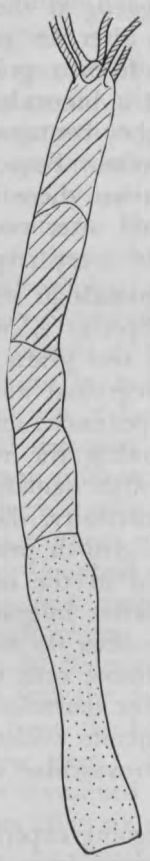

FIG. 3.

This figure illustrates schematically the interposition of three gastric regions of Species X (cross lines) between a graft consisting of the proximal region of *P. oligactis* (stippled area) and the distal region (hypostome, tentacles, and growth region) of Species X.

sumably pass into the gastrodermis where they, plus their enclosed nematocysts, are digested. Obviously, more experimentation is necessary before the nature of this inhibition is elaborated.

In order to determine whether the inhibitory substance diffused from cell to cell or reached the tissues of Species X via the gastrovascular cavity, the following experiment was performed. The proximal portion of *oligactis* was grafted to a distal region of Species X, but on this occasion the gastric regions of 3 normal X species were inserted between the two grafted portions (See Figure 3). It was assumed that if the inhibitory substance diffused from cell to cell there would be little or no inhibition of nematocyst formation evidenced after a period of six days, because the substance would have to pass through the gastric regions of 3 animals before exerting its influence upon the cnidoblast cells of Species X. On the other hand, it would be expected that if the migration took place via the gastrovascular cavity, where continual flagellar currents are in operation, that there would be a very noticeable inhibition of nematocyst formation after a period of six days. Ten animals examined after six days could not be distinguished from normal forms in regard to nematocyst distribution. Both the growth regions and all regions of the tentacles contained an abundant supply of this organelle. Since the grafts begin to fragment after six days it was impossible to study them for longer periods of time.

It was also desired to determine in what body region of *oligactis* the inhibitory substance had its origin. Five proximal regions (including only the peduncle and basal disk of *oligactis*) were grafted to distal portions of Species X. These grafts were examined daily under an oil immersion objective for a period of eight days. There was no noticeable inhibition of nematocyst formation. After eight days these grafted animals were stained with methylene blue buffered at p.H 7 and mounted as whole animals. There was a regular, uniform distribution of nematocysts from the bases to the extremities of the tentacles at this time. Therefore, it appears quite obvious that the inhibitory substance has its origin in the stomach or gastric region of the animal.

Finally, 8 animals consisting of a graft of proximal portions of *oligactis* to distal portions of Species X were sectioned for histological study. Two individuals were fixed in Bouin's solution on the first day after the graft was performed; additional groups of 2 animals were fixed on the 3rd, 4th, and 6th days It was not until the fourth day that nematocyst inhibition was recognized with certainty. At this time, interstitial cells transforming into cnidoblasts, and cnidoblasts forming inter-

stitial cells are evident, but the number of these cells as compared with animals examined on the first day is greatly reduced. Moreover, it was noted that very few or no nematocysts were being formed in the growth region; practically all the nematocysts sighted in the sections were located near the junction of the two grafted portions. Animals examined on the sixth day contained virtually no cnidoblast cells in the growth region, but occasional nests of cnidoblast cells were found in the region just proximal to the growth region.

It is difficult to interpret the significance of the foregoing observations. Obviously, some substance or substances in the gastric region of *P. oligactis* are capable of inhibiting the formation of nematocysts in Species X to a great extent. The fact that this inhibition is much more evident in the growth region of Species X than in regions proximal to the growth region cannot be explained on the basis of present evidence.

It is not surprising to find that the majority of nematocysts in Species X are located at the junction of the grafted portions. Since there is no large scale tissue exchange between the two species, nematocysts proximal to the growth region will be forced through growth processes towards the junction of the grafted portions. The nematocysts will accumulate in this area as long as new nematocysts are being formed proximal to the growth region and as long as normal growth processes are in operation. However, it must be stressed that even in regions proximal to the growth region of Species X, there is a strong inhibition of nematocyst formation when the interstitial cells in this region are under the influence of the tissues of *P. oligactis*.

It may be mentioned briefly that if the grafting procedure is reversed, i.e., if the distal portion of *oligactis* is grafted to the proximal portion of Species X, there is no apparent inhibition of the nematocyst formation process. However, there are, after such a graft, very interesting growth reversals in *oligactis*, but these results will be the subject of another publication.

The present experiments have offered strong evidence to support the theory that nematocysts in hydra are capable of migrating via the gastrovascular cavity from the gastric region to functional positions in the nematocyst batteries of the tentacles. Also there is a strong indication that nematocysts normally move to the into the tentacles from the growth region where they are formed because of force exerted on them by actively dividing cells in this region.

It has also been demonstrated that *P. oligactis* possesses in the tissues of its gastric region a substance or substances which are capable of inhibiting the differentiation of interstitial cells into cnidoblast cells. This substance apparently diffuses from cell to cell rather than passing through the gastrovascular cavity. Furthermore, this substance does not produce a permanent inhibition of nematocyst formation because if the source of the inhibitory substance is removed, differentiation of interstitial cells into cnidoblasts begins within a very short time.

It is hypothesized that the same substance which inhibits the development of nematocysts in Species X also plays a very important role in controlling the differentiation of interstitial cells in the tissues of *P. oligactis*. Attempts are being made at the present to prepare an extract of *oligactis* which will elicit the same response as the entire animal does when grafted to the tissues of Species X.

Summary.

A technique has been described whereby it is possible to inhibit to a very large extent the process of nematocyst formation in hydra. *Pelmatohydra oligactis* possesses a substance in its stomach region, which, after passing into the cells of another species of hydra, is capable of inhibiting the transformation of interstitial cells into cnidoblast cells. The nature of this substance, or possibly substances, is not known at the present.

It has been clearly demonstrated that nematocysts in hydra located in the growth and gastric regions are capable of passing to their final position in the tentacle batteries through the gastrovascular cavity. Normally, however, nematocysts are forced into the tentacles from the growth region because of an active multiplication of cells in this region, hence an epidermal migration.

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